IONIC BALANCE IN BLOOD AND COELOMIC FLUID OF EARTHWORMS[1]

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The osmotic relations in earthworms have been studied by several investigators and have been reviewed by Ramsay (1949a). It is well established that earthworms behave osmotically like fresh-water animals. Water enters the body through the skin and is eliminated by the nephridia with the production of a hypotonic urine (Bahl, 1947; Ramsay, 1949a, 1949b).

Only sporadic reports are available on the ionic balance in earthworms. Bahl (1947) reported the ionic concentrations of the body fluids of *Pheretima posthuma,* indicating that the sodium concentration of the coelomic fluid was twice as concentrated as that of the blood. He also showed that the chloride concentration of the blood was lower than that of the coelomic fluid, the concentration being only about a third of that of the sodium concentration. Ramsay (1949a) reported the concentration of chloride in the blood and coelomic fluid of *Lumbricus terrestris.* He showed that the chloride concentration of the blood was slightly lower than that of the coelomic fluid. Prosser and Brown (1961) cite values for sodium and potassium in the body fluids of *Lumbricus,* showing a higher concentration of sodium and a lower concentration of potassium in blood as compared to coelomic fluid.

This paper is concerned with the balance of sodium, potassium, calcium and chloride in the blood and coelomic fluid of earthworms.

Materials and Methods

Three species of earthworms were used in this study. *Lumbricus terrestris* was obtained from the Carolina Biological Supply Co. *Eisenia foetida* was collected from manure compost on campus, while *Helodrilus caliginosus* was obtained locally from bait dealers. All animals were kept either in moist soil or aerated tap water in large aquaria for a week before being used in any of the experiments. The animals were kept at 16° C. and all experiments were carried out at this temperature. Only large adult worms were used.

Blood and coelomic fluid were collected in the following manner. A drawn capillary was inserted directly into the coelom through the body wall and the coelomic fluid was readily collected. The earthworm was then anesthetized individually with 15% ethanol. It was found that anesthetizing the animal does not affect the ionic concentration of the blood. The body wall was then cut dorsally and pinned out on a waxed pan to expose the dorsal blood vessel and aortic arches. The coelomic fluid was blotted away and a drawn capillary was inserted into either the dorsal blood vessel or aortic arch. By this method, it was possible to collect

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a sample of blood sufficient for the analyses of the cations and chloride. Blood was collected only from *L. terrestris* and *H. caliginosus.* Because of the size of *E. foetida,* blood was not collected from this species.

Samples of blood and coelomic fluid were centrifuged and the supernatant fluid was analyzed for sodium, potassium and calcium with a Coleman flame photometer. Chloride was determined with an Aminco-Cotlove chloride titrator.

Body fluids of animals kept in moist soil, tap water and 0.1 *M* NaCl solution were analyzed for ionic concentrations in *L. terrestris* and *E. foetida. H. caliginosus* from moist soil was studied for comparison.

Results

The results are presented in Table I. The concentrations of sodium, potassium and calcium are significantly higher in the blood than in the coelomic fluid in all groups studied. There is no difference in the concentrations of chloride in the body fluids. The chloride concentration is about 50% of the sodium concentration. With exposure to 0.1 *M* NaCl solution, the concentration of sodium in both the blood and coelomic fluid is increased and maintained higher than the concentration in the medium. There is almost no difference in the ionic concentrations in the three species studied under the same conditions.

Discussion

The data presented in this study demonstrate that there is regulation of sodium, potassium and calcium concentrations between the blood and coelomic fluid of earthworms. The concentrations of these ions are always greater in the blood than in the coelomic fluid. This relationship has been pointed out most recently by Prosser and Brown (1961), who have cited a higher value for sodium in the blood than in the coelomic fluid of *Lumbricus.* However, a higher value for potassium is cited in the coelomic fluid. No experimental details were presented. Bahl (1947) reported a concentration of sodium in the coelomic fluid twice that in the blood of *Pheretima posthuma.* His value for sodium in coelomic fluid (80 meq./l.) is comparable to the values for the three species reported in this paper. He also reported a higher concentration of calcium and chloride in the coelomic fluid but lower values of potassium as compared to blood. There is no difference in the concentration of chloride between the body fluids studied in *H. caliginosus* and *L. terrestris.* Ramsay (1949a) compared the chloride concentrations in two animals of *L. terrestris* and reported that the blood chloride was slightly lower than in the coelomic fluid. His values (43 and 46 meq./1. in blood and coelomic fluid, respectively) are comparable to the values obtained for this species in this study.

The sodium concentration of the body fluids is maintained higher than that of the environment when the animal is placed in 0.1 *M* NaCl, a concentration greater than that of the body fluids of worms in soil or tap water. Ramsay (1949a) has shown that although the osmotic pressure of the coelomic fluid, as measured by the freezing point method, remains higher than the concentration in the medium as high as 0.24 *M* NaCl, the chloride concentration becomes hypotonic to the medium at an external concentration of 0.055 *M* NaCl. The concentration of sodium is

TABLE I

Ionic concentrations in blood and coelomic fluid of earthworms

	Ionic conc. (meq./l.)						
	No. animals	Coelomic fluid		No. animals	Blood		p
		Mean	SD		Mean	SD	
Lumbricus terrestris							
Moist soil							
Na	7	75.6	4.5	7	85.7	6.6	.01
K	7	4.0	1.5	7	5.5	1.0	.05
Ca	7	5.9	1.7	7	16.7	3.1	.001
Cl	7	42.8	2.6	10	39.0	8.3	.20
Tap water							
Na	24	78.1	2.9	20	83.6	3.5	.001
K	24	2.7	0.7	20	5.3	1.3	.001
Ca	23	4.6	1.1	8	12.2	1.4	.001
Cl	16	48.5	4.8	14	47.2	4.7	.70
0.1 *M* NaCl							
Na	10	112.7	3.6	8	122.1	10.1	.02
K	10	3.3	0.5	8	7.0	0.8	.001
Ca	10	8.3	1.3	5	14.8	1.1	.001
Cl	10	74.4	6.2	8	77.7	11.2	.70
Helodrilus caliginosus							
Moist soil							
Na	14	78.6	5.1	10	88.5	7.7	.001
K	14	5.8	1.6	10	7.9	0.8	.001
Ca	14	7.3	1.6	10	26.7	6.5	.001
Cl	15	35.6	3.0	8	35.9	2.7	.80
Eisenia foetida							
Moist soil							
Na	20	79.7	4.8				
K	20	4.0	0.9				
Ca	20	8.0	2.1				
Cl	20	42.0	6.7				
Tap water							
Na	16	70.0	7.3				
K	16	3.5	1.1				
Ca	16	5.6	0.8				
Cl	10	42.0	6.7				
0.1 *M* NaCl							
Na	12	116.5	3.9				
K	12	3.6	0.8				
Ca	12	6.8	2.6				
Cl	11	88.4	3.6				

approximately twice that of chloride in the body fluids. This would mean then that the earthworm can maintain hypertonicity with respect to sodium to 0.11 *M* NaCl in the medium. It is shown in Table I that sodium in the body fluids of earthworms remains hypertonic to the medium at an external concentration of 0.1 *M* NaCl.

It is interesting to note that the chloride of both the blood and coelomic fluid is about 50% of the concentration of sodium. This phenomenon has been reported by Bahl (1947) for *Pheretima posthuma*. Maluf (1940) showed that the chloride concentration of a mixture of blood and coelomic fluid (46 meq./l.), considered as NaCl, can account for only 29% of the total osmotic pressure of this mixture. Ramsay (1949b) reported that NaCl, based on chloride concentrations, can account for about 50% of the total osmotic pressure of the coelomic fluid, assuming that sodium and chloride form a large proportion of inorganic substances present. The sodium concentration was not determined. Both authors suggest that organic substances are responsible for the remaining fraction of the total osmotic pressure. The osmotic pressure of tap water animals is given as 90 meq. NaCl/1. (Ramsay 1949a). It should be noted that the total concentration of the three cations studied in the coelomic fluid of tap water animals is 85 meq. (Na, K, Ca)/1. It seems probable that the inorganic ions do contribute to most of the total osmotic pressure in earthworm body fluids. Anions other than chloride then must be responsible for total electrolyte balance.

Summary

1. The concentrations of sodium, potassium, calcium and chloride in the blood and coelomic fluid were determined in *Lumbricus terrestris* kept in moist soil, tap water and 0.1 *M* NaCl solution, and in *Helodrilus caliginosus* kept in moist soil. Coelomic fluid only was analyzed in *Eisenia foetida*.

2. The concentrations of cations are higher in the blood than in the coelomic fluid in all groups studied. There is no difference in the concentration of chloride between the blood and coelomic fluid.

3. Ionic concentrations are similar in the three species studied under similar conditions.

4. The concentration of sodium in both blood and coelomic fluid is maintained greater than the medium in 0.1 *M* NaCl solution.

LITERATURE CITED

Bahl, K. N., 1947. Excretion in the Oligochaeta. *Biol. Rev.*, 22: 109–147.

Maluf, N. S. R., 1940. Osmo- and volume-regulation in the earthworm with special reference to the alimentary tract. *J. Cell. Comp. Physiol.*, 16: 175–187.

Prosser, C. L., and F. A. Brown, Jr., 1961. Comparative Animal Physiology, Second Edition. W. B. Saunders Company, Philadelphia.

Ramsay, J. A., 1949a. The osmotic relations of the earthworm. *J. Exp. Biol.*, 26: 46–56.

Ramsay, J. A., 1949b. The site of formation of hypotonic urine in the nephridium of *Lumbricus*. *J. Exp. Biol.*, 26: 65–75.